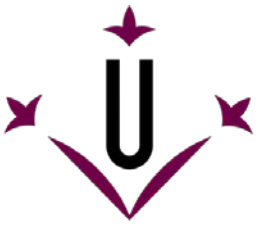

LONG LIFESPANS HAVE EVOLVED WITH LONG AND MONOUNSATURATED FATTY ACIDS IN BIRDS

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1 table

2 figures

Abstract

The evolution of lifespan is a central question in evolutionary biology, begging the question why there is so large variation among taxa. Specifically, a central quest is to unravel proximate causes of ageing. Here we show that the degree of unsaturation of liver fatty acids predicts maximum lifespan in 107 bird species. In these birds, the degree of fatty acid unsaturation is positively related to maximum lifespan across species. This is due to a positive effect of monounsaturated fatty acid content, while polyunsaturated fatty acid content negatively correlates with maximum lifespan. Furthermore, fatty acid chain length unsuspectedly increases with maximum lifespan independently of degree of unsaturation. These findings tune theories on the proximate causes of ageing while providing evidence that the evolution of lifespan in birds occurs in association with fatty acid profiles. This finding suggests that studies of proximate and ultimate questions may facilitate our understanding of these central evolutionary questions.

Introduction

The evolution of lifespan is a central question in evolutionary biology (Williams et al. 2006, Lohr et al. 2014). While many theoretical and empirical studies have addressed the evolution of lifespan, this literature has emerged almost in complete isolation from the evolution of the underlying mechanisms. Already Tinbergen (1963) emphasized the importance of investigating ontogeny, mechanisms, function and evolution, but also that these questions are complementary rather than mutually exclusive. Thus when studying the evolution of a specific character such as longevity it might be highly illuminating to also study the evolution of the underlying mechanisms. In other words, it may only be possible to understand the evolution of a character by simultaneously investigating the evolution of the mechanisms that produce divergent characters among taxa. Most organisms deteriorate as they age and consequently die, still longevity varies enormously among species (Finch 1990), perhaps evolving in response to variation in extrinsic mortality (Austad 1993, Reznick et al. 2004). The emerging evolutionary theories of ageing, as opposed to the increasingly outdated but still mainstream theories, posit that natural selection generates mechanisms that promote ageing in cells and long-lived animals have lower levels of proageing factors such as low mitochondrial rates of reactive oxygen species (ROS) generation and low degrees of fatty acid unsaturation (Pamplona and Barja 2007, 2011). However, an increasing body of conflicting results (Speakman and Selman 2011, De Loof et al. 2013, Gladyshev 2014, Valencak and Azzu 2014) points to the necessity to test these theories in comparative studies of different species (Valencak and Azzu 2014).

A relationship between membrane fatty acid composition and longevity, termed the homeoviscous longevity adaptation hypothesis or membrane pacemarker theory, has received support and represents a cornerstone for understanding the ageing process and the evolution of lifespan (Pamplona et al. 2002, Hulbert et al. 2007, Pamplona and Barja 2007, 2011). The homeoviscous longevity adaptation hypothesis is based on the fact that double bonds in fatty acid aliphatic chains of cell membrane lipids are separated by methylene groups that increase the risk of

lipid peroxidation, which theoretically increases with the number of double bonds, being higher in polyunsaturated fatty acids (PUFAs) than in monounsaturated fatty acids (MUFAs) and saturated fatty acids (Hulbert et al. 2007, Pamplona 2008). A consequence of lipid peroxidation is the generation of reactive carbonyl species including α,β-unsaturated aldehydes whose non-charged structure allows them to migrate far from the production sites to react with nucleophilic groups of a range of macromolecules and produce cytotoxic advanced lipoxidation end products, which ultimately cause the loss of cellular function (Pamplona 2008).

The homeoviscous longevity adaptation hypothesis then predicts that organisms with a lower degree of membrane fatty acid unsaturation will live longer (Pamplona and Barja 2007, 2011, Pamplona et al. 2002, Hulbert et al. 2007, Pamplona 2008). However, it is also known that the kink in the fatty acyl chain that is produced when a first double bond is added increases membrane fluidity, necessary for cell functionality, while that does not happen when additional double bonds are incorporated (Brenner 1984). Thus, it could also be predicted that the content of MUFAs in membrane fatty acids should increase longevity, as some comparative data indeed suggest (Buttemer et al. 2008), with the possibility that a high MUFA content in long-lived organisms gives rise to a high total content of double bonds. In that case, a high degree of unsaturation would be indicative of a fatty acid pattern resistant to peroxidation. Furthermore, peroxidation susceptibility may also depend on the nature of the microenvironment in which fatty acids react with oxidants, which can cause some PUFAs to not favour autooxidation and even exert antioxidant effects (Richard et al. 2008). Therefore, it seems clear that membrane composition determines cell functionality, but the exact characteristics of fatty acids that affect longevity are unknown, which in turn prevents the formulation of specific predictions about longevity of different organisms. In addition, some findings do not support the homeoviscous longevity adaptation hypothesis because of a lack of associations between fatty acid composition and longevity in both intraspecific and interspecific studies (Valencak and Azzu 2014). As a consequence, a more detailed insight into the

relationship between fatty acid profile and longevity is needed to advance our understanding of the evolution of lifespan.

To demonstrate that fatty acid composition is a general contributing factor of the rate of ageing and, consequently, longevity determination, extensive comparisons among species across a large phylogenetic spectrum are necessary. This has never been achieved. About a maximum of 40 mammal species have been tested for an association between fatty acid composition and longevity (Valencak and Ruf 2007). Other comparative investigations have only been investigated a few distantly related clusters of taxa, making it difficult to separate the effect of fatty acid composition from that of common ancestry in explaining variation in longevity. In these studies, multiple independent statistical tests and severe collinearity between fatty acid characteristics constitute further problems for the validation of the homeoviscous longevity adaptation hypothesis (Rice 1989). Importantly, it is unknown whether it is the number and position of double bonds in the fatty acid aliphatic chain, chain length, or a combination of these factors that determine susceptibility to lipid peroxidation (Di Nunzio et al. 2011). Therefore, we investigated the relationship between liver fatty acid composition and maximum lifespan in 107 species of birds with longevities ranging from 5 to 44 years (electronic supplementary material, Table S1), varying in body size over a wide range (nearly 650-fold) and belonging to 16 taxonomic orders ranging from Galliformes to Passeriformes and thus covering the entire phylogenetic spectrum of the class Aves (Jarvis et al. 2008). To determine the species-specific information, we sampled 487 individual birds. We were able to distinguish the effects exerted by individual fatty acid characteristics and determine their relative importance in explaining variation in maximum lifespan among species by using partial least squares regression models (Carrascal et al. 2009).

Materials and methods

BODY MASS AND LIVER SAMPLING

All tissue samples were collected from fresh specimens brought by the general public to J.E. during 2001-2012. All specimens were individually numbered and recorded in an official protocol in accordance with Danish legislation. All specimens came from natural populations, most from Southern Jutland, Denmark, with fewer birds from other parts of Denmark. On the basis of standard plumage characteristics, we could assign an age class (adult or subadult) to a total of 372 birds, from which 196 were adults and a similar number (176) were subadults (electronic supplementary material, Table S1). The mean ratio adults:subadults, considering the species in which an age class could be assigned to more than two birds, is 0.5 (Table S1). Although we could not identify the age class of all birds, this suggests that our database likely comprises a balanced proportion of adult and subadult birds. Therefore, it is not likely that our results are biased by the age of the specimens.

Birds were first weighed on a precision balance to the nearest 0.1 g before being opened and a small piece of the liver being placed in an eppendorf tube before being frozen at -80 ºC. A total of 14 specimens with miscoloured livers or with clearly visible tumors in their liver were excluded from the samples.

MAXIMUM LIFESPAN ESTIMATES

We obtained information on maximum lifespan of most (80%) species from The Animal Ageing and Longevity Database (AnAge; Tacutu et al. 2013). For six of the species included in our study, the sampling effort to calculate maximum lifespan in AnAge is low (10-100 recoveries; electronic supplementary material, Table S1). As reliable information on maximum lifespan is only obtained with high sampling effort (Møller 2006), we used the lifespan estimates provided by AnAge for species with sampling effort larger than 100 recoveries, and used the lifespan estimates provided by the European bird ringing organization EURING (http://www.euring.org) for the other six species (sampling effort: 518-21,370 recoveries, Table S1). Thus, our lifespan estimates are not biased by sampling effort as all were based on large numbers of recoveries. We also used the

lifespan estimates provided by EURING, all obtained from long-term natural populations of banded birds, for another 14 species (Table S1) for which AnAge only has information on captive birds or the origin of birds used to estimate lifespan (wild vs. captivity) is unknown, as animal lifespan is considerably greater in captivity than in the wild due to the absence of extrinsic causes of mortality (Ricklefs and Scheuerlein 2001). Therefore, we only used lifespan estimates calculated from natural populations. Lastly, we used the lifespan provided by EURING for the Short-toed Tree-creeper *Certhia brachydactyla*, as there is no information available for this species in AnAge.

SAMPLE PROCESSING

A quantity of 100 mg of liver were homogenized separately in a buffer containing 180 mM KCl, 5 mM 3-[N-morpholino]propanesulfonic acid, 2 mM ethylenediaminetetraacetic acid (EDTA), 1 mM diethylenetriaminepentaacetic acid and 1 mM butylated hydroxyl toluene, 10 mg/mL aprotinin, 1 mM phenylmethylsulfonyl fluoride, pH 7.3 with a Potter–Eljeveim device at 4 °C. Protein concentration was measured using the Bradford assay (Bio-Rad Laboratories, Germany) with bovine serum albumin as a standard. Total lipids from tissue samples were extracted with chloroform:methanol (2:1, v/v) in the presence of 0.01% butylated hydroxytoluene to avoid artifactual oxidation.

FATTY ACID COMPOSITION

Fatty acyl groups were analysed as methyl esters derivatives by gas chromatograpgy (GC). Briefly, fatty acids were transesterified by incubation in 2 ml of 5 % methanolic HCl at 75 ºC for 90 min. The resulting fatty acid methyl esters (FAMEs) were extracted by adding 2 ml of n-pentane and 1 ml of saturated NaCl solution. The n-pentane phase was separated, evaporated under nitrogen, redissolved in 80 µl of carbon disulfide and 2 µl were used for GC analysis. The analysis was performed on a GC System 7890A with a Series Injector 7683B and a FID detector (Agilent

Technologies Inc., Barcelona, Spain) equipped with a DBWAX capillary column (length 30 m x inner diameter 0.25mm x film thickness 0.20 μm; Agilent Technologies Inc.). The injections were performed in the splitless mode. The temperature of the injector was 220 ºC. The flow rate of helium (99.99 %) carrier gas was maintained at a constant rate of 1.8 mL/min. The column temperature was held at 145 ºC for 5 min; subsequently, the column temperature was increased by 2 ºC/min to 245 ºC for 50 min, and held at 245 ºC for 10 min, and with a post-run of 250 ºC during 10 min. Identification of FAMEs was made by comparison with authentic standards (Larodan Fine Chemicals, Malmö, Sweden). Results were expressed as mol %. The following fatty acid indexes were calculated: saturated fatty acids (SFA); unsaturated fatty acids (UFA); monounsaturated fatty acids (MUFA); polyunsaturated fatty acids from n-3 and n-6 series (PUFAn-3 and PUFAn-6); average chain length (ACL) = [(Σ%Total14 x 14) + (Σ%Total16 x 16) + (Σ%Total18 x 18) + (Σ%Total20 x 20) + (Σ%Total22 x 22)+ (Σ%Total24 x 24)]/100]; double bond index (DBI) = [(1 x Σmol% monoenoic) + (2 x Σmol% dienoic) + (3 x Σmol% trienoic) + (4 x Σmol% tetraenoic) + (5 x Σmol% pentaenoic) + (6 x Σmol% hexaenoic)]; peroxidizability index (PI) = [(0.025 x Σmol% monoenoic) + (1 x Σmol% dienoic) + (2 x Σmol% trienoic) + (4 x Σmol% tetraenoic) + (6 x Σmol% pentaenoic) + (8 x Σmol% hexaenoic)]; and anti-inflammatory index (AI): [[(20:3n-6) + (20:5n-3) + (22:6n-3)]/ (20:4n-6)]*100.

We used the mean value per species of fatty acid composition variables. The within-species repeatability (Møller and Birkhead 1994) of all fatty acid composition indices, calculated for those species with a sample size of at least two individuals, was statistically significant (ACL: $F_{63,381}$ = 3.94, r = 0.30; SFA, $F_{63,381}$ = 2.42, r = 0.17; UFA: $F_{63,381}$ = 2.70, r = 0.20; MUFA: $F_{63,381}$ = 5.33, r = 0.39; PUFA: $F_{63,381}$ = 5.67, r = 0.41; PUFAn-3: $F_{63,381}$ = 4.26, r = 0.32; PUFAn-6: $F_{63,381}$ = 7.30, r = 0.48; DBI: $F_{63,381}$ = 3.70, r = 0.28; PI: $F_{63,381}$ = 3.62, r = 0.28; AI: $F_{63,381}$ = 7.09, r = 0.47; all P < 0.0001). This indicates that bird species are consistent in their fatty acid composition and allows the use of species means in comparative analyses (Møller and Birkhead 1994).

STATISTICAL ANALYSES

We analysed the relationship between the response variable (maximum lifespan) and the mean fatty acid composition indices per species (ACL, SFA, UFA, MUFA, PUFA, PUFAn-3, PUFAn-6, DBI, PI and AI; predictor variables) by means of partial least squares regressions (Carrascal et al. 2009). We also added the mean body mass of species (as a surrogate of body size) as a predictor to avoid detecting effects of fatty acid composition on maximum lifespan that may arise because of associations between body mass and maximum lifespan (de Magalhães et al. 2007, Valencak and Azzu 2014). Although fatty acid characteristics may be affected by developmental and growth rates of species (de Magalhães et al. 2007), body size is strongly positively correlated with growth rate in birds (Ricklefs 1968). Thus, by controlling by body size of species we also control for any potential confounding effect of growth rate.

We made another partial least squares regression model including the proportion of each of the 18 fatty acids considered instead of the fatty acid composition indices to explore the contribution of individual fatty acids to explain variance in maximum lifespan across species. All variables were $\log_{10}$ transformed to ensure normality assumptions, except the proportion of individual fatty acids, which was arcsine square-root transformed. An additional partial least squares regression model excluding the species for which only one individual could be sampled (N = 43, electronic supplementary material, Table S1) provided virtually identical results as when using the entire dataset (Table 1), indicating that our results were not dependent on sample size per species.

Partial least squares regression is an extension of multiple regression analysis in which associations are established with components extracted from predictor variables that maximize the explained variance in the response variable. These components are defined as a linear combination of predictor variables, so the original multidimensionality is reduced to a small number of orthogonal components to detect structure in the relationships between predictor variables and between these factors and the response variable. The extracted components account for

successively lower proportions of original variance. The relative contribution of each predictor variable to the derived components is provided by the square of the predictor weight (Carrascal et al. 2009). Results obtained with partial least squares regression are similar to those from conventional multiple regression techniques. However, this method is extremely resilient to the effects of sample size and degree of correlation between predictor variables, which makes partial least squares regression especially useful when sample size is small and in cases of severe multicollinearity (Carrascal et al. 2009). There is a significant correlation among fatty acid composition indices (mean absolute Pearson's correlation coefficient: $r = 0.46$, $N = 107$, $P < 0.0001$) and among the proportions of individual fatty acids ($r = 0.21$, $N = 107$, $P = 0.028$), which makes partial least squares regression the most appropriate analytical tool for investigating effects of fatty acid composition on maximum lifespan.

We only considered the first partial least squares regression component extracted, whose significance was determined by testing the significance of the correlation coefficient of the relationship between partial least squares regression scores for maximum lifespan and partial least squares regression component scores, thus determining if the amount of variance explained in maximum lifespan was significant. We also determined the contribution of predictors to the partial least squares regression model, which was made by testing the statistical significance of the regression coefficients of the predictors, thus determining the degree of correlation between the response variable and these predictors. The latter test was made by bootstrapping using 1000 replicates (Galván et al. 2014). Partial least squares regression analyses were made with STATISTICA 12.0 (StatSoft, Inc., Tulsa, OK, USA) and TANAGRA 1.4 (Rakotomalala 2005).

PHYLOGENETIC ANALYSES

Bird species are evolutionarily related through their common phylogenetic history, which can lead to overestimation of degrees of freedom if phylogenetic relationships are not taken into account (Felsenstein 1985). We used phylogenetic eigenvector regression to correct for the effect of

common ancestry in the analysis of the relationship between maximum lifespan and fatty acid composition (Diniz-Filho et al. 1998, Galván et al. 2014). Phylogenetic eigenvector regression is based on the eigenfunction decomposition of phylogenetic distance matrices, so that phylogenetic relationships between species can be translated into predictor variables (phylogenetic eigenvectors) that capture phylogenetic effects (Diniz-Filho et al. 1998). To obtain the eigenvectors, we performed a principal coordinates analysis on the matrix of pairwise phylogenetic distances between the 107 bird species (after a double-centre transformation) using MVSP 3.22 (Covach Computer Services, Pentraeth, UK). Eigenvectors extracted from such distance matrices detect the main topological features of the cladogram under different sample sizes or number of taxa used in the analyses (Diniz-Filho et al. 1998).

Partial least squares regression can be used when the number of predictors is similar to sample size while still avoiding overfitting (Carrascal et al. 2009), and the description of the phylogenetic relationships between species is most efficient when the total number of phylogenetic eigenvectors is used (Rohlf 2001, Diniz-Filho et al. 2012). Therefore, we used the first 73 phylogenetic eigenvectors extracted, which account for 99 % of phylogenetic structure in the phylogenetic distance matrix (or 50 phylogenetic eigenvectors accounting for 98 % of phylogenetic structure in the reduced model excluding species with sample size of one individual). The extracted phylogenetic eigenvectors can be used as predictor variables in any other statistical linear model to correct for phylogenetic effects on response variables, and thus we used the phylogenetic eigenvectors obtained as additional predictors in the partial least squares regression models described above.

To make the phylogenetic hypothesis for our species of birds, we used a species-level supertree constructed for relationships below the order level (Galván et al. 2014), and the recent genome analysis by Jarvis et al. (2014) for relationships between Orders (Fig. 1). We assumed that all branch lengths were equal to unity.

Results

Considering all fatty acid characteristics together with body size of the species and phylogenetic effects as explanatory variables in a partial least squares regression model, we obtained a component that explained a very large amount (65.7%, $P < 0.0001$, Table 1) of the observed variance in maximum lifespan across species, with a positive component associated with maximum lifespan ($r = 0.81$, $P < 0.0001$, Fig. 2). As the square predictor weights represent the relative contribution of predictor variables to the derived model component, it can be determined that body mass, all fatty acid composition variables as a whole and phylogeny independently explain 17.1%, 18.8% and 5.1%, respectively, of variance in maximum lifespan across species. The model shows that irrespective of body size and phylogenetic effects, fatty acid chain length, the proportion of MUFA and double bond and peroxidizability indices are positively correlated with maximum lifespan across species, while the proportion of total PUFAs and PUFAn-6 and the anti-inflammatory index negatively correlate with maximum lifespan (Table 1). The most important feature of these fatty acids is chain length, accounting for 7.8% of the total variance explained by the model, while the other fatty acid characteristics account for between 0.02 and 3.7% of model variance (Table 1).

Our study thus reveals that variation in maximum lifespan among species is mainly determined by fatty acid chain length independent of the degree of unsaturation. Indeed, maximum lifespan increases with increasing proportion of highly unsaturated but long-chain fatty acids such as adrenic (C22:4n-6) and docosapentaenoic (C22:5n-6) acids, but the proportion of a saturated and medium-chain fatty acid such as the myristic acid (C14:0) shows a tendency, albeit non-significant, to be negatively correlated with maximum lifespan (Table 1). Although opposite to predictions made by the homeoviscous longevity adaptation hypothesis, the positive and independent effects of double bond and peroxidizability indices on maximum lifespan were due to the positive effect of MUFA content on maximum lifespan, indicating that the degree of unsaturation is actually positively associated with longevity because long-lived species have more MUFAs, suggesting that

303 the resistance to lipid peroxidation is an optimized feature associated with bird longevity. As an
304 extension of this evolutionary adaptive response, long-lived birds also showed a lower anti-
305 inflammatory index, likely due to a higher cell resilience to somatic stressors (Finch et al. 2010).

306

307 ## Discussion

308 We suggest that the addition of one double bond to fatty acid chains contributes to increased
309 longevity. Although we made our analyses on total liver lipids, a large proportion of these must
310 necessarily be membrane lipids. It is therefore likely that the effects that we found on lifespan are at
311 least partly exerted through the known influence of cell membrane fatty acids on membrane
312 properties. Thus, it is well known that maintaining a certain membrane fluidity is essential for
313 cellular function (Shinitzky 1984). Adding one double bond triggers fluidity, but multiple double
314 bonds increase membrane permeability (Brand et al. 1994) without additional increase in membrane
315 fluidity (Brenner 1984) while increasing the susceptibility to lipid peroxidation (Pamplona 2008).
316 Thus, lipid characteristics, i.e. increased monounsaturates and decreased polyunsaturates, that
317 maximize membrane fluidity without compromising protection against peroxidation are found in
318 species that live longer. Previous information on some species of birds suggested that larger birds
319 have more monounsaturated and less polyunsaturated fatty acids in liver mitochondria (Brand et al.
320 2003). As body size is positively associated with longevity in birds (Table 1), our results agree with
321 those previous findings, suggesting that increasing monounsaturation and decreasing
322 polyunsaturation of fatty acids may be a general strategy that has evolved together with increased
323 longevity. In this regard, a recent study (Jobson et al. 2010) with a phylogenomic approach to
324 identify genetic targets of natural selection for increased longevity in mammals shows that genes
325 involved in lipid composition have collectively undergone increased selective pressure in long-lived
326 species, reinforcing the suggestion that cell membrane has been an optimized feature during
327 evolution (Pamplona 2008, Naudí et al. 2013).

328 More importantly, we reveal that, irrespective of unsaturation effects, having long-chain
329 fatty acids is an important strategy to achieve high longevity. In vertebrates, fatty acid chain length
330 of cell membranes is strictly maintained around 18 C atoms (Pamplona 2008), and in agreement,
331 average chain length of our model species ranged from 17 to 19 C atoms (electronic supplementary
332 material, Table S1). However, our findings show that it is the relative contribution of longer-chain
333 fatty acids to the total fatty acid content that better explains variation in lifespan. This independent
334 effect of fatty acid chain length on longevity had not been suspected before. It must be noted,
335 however, that although fatty acid characteristics can partly explain interspecific variation in
336 maximum lifespan, they are not the only factors that affect lifespan. Specifically, for example, it is
337 known that proteins from short-lived species have higher methionine content than those from long-
338 lived species, probably because the antioxidant capacity of methionine selects for the addition of
339 this amino acid to proteins in short-lived animals subject to higher oxidative stress (Pamplona et al.
340 2005, Pamplona and Barja 2007, Aledo et al. 2011). Future comparative studies should investigate
341 how the evolution of fatty acid characteristics and protein methionine contents interact with
342 oxidative stress levels to explain variation in animal lifespan.

343 Cell membranes are dynamic structures that require continuous adjustments in the chemical
344 composition and molecular shape of their lipid constituents, particularly of the phospholipids
345 (Mouritsen 2005, McMahon and Gallop 2005, Hulbert et al. 2014). A major factor determining the
346 shape of phospholipids is the nature of their hydrophobic tail, the fatty acid residues. At
347 physiological temperatures, the length of a phospholipid molecule is directly proportional to the
348 number of C atoms and inversely proportional to the number of double bonds present in its fatty
349 acid chains. In addition, the molecular shape of a phospholipid within the bilayer is ultimately
350 determined by the compatibility between the size of its polar head group and that of its hydrophobic
351 tail. Thus, the average chain length and the degree of unsaturation are determinants of the
352 membrane lipid geometry that, in turn, have major consequences on the functional properties of
353 cells. Therefore, the mechanism by which increased membrane fatty acid chain length benefits

longevity has to be investigated, although it is likely that a high proportion of long-chain fatty acids helps avoid lipid peroxidation.

Variation in lifespan across taxa is assumed to be caused by environmentally-mediated mortality, with high mortality rates leading to high senescence rates and short lifespan (Austad 1993, Reznick et al. 2004). It is believed that extrinsic mortality affects the evolution of lifespan by pleiotropic effects: high mortality rates promote rapid reproduction, and direct selection for rapid reproduction leads to indirect selection for shorter lifespan (Williams 1957). However, a more complex scenario for the evolution of lifespan that considers that senescence is adaptive in certain circumstances has recently emerged (Longo et al. 2005, Mitteldorf and Martins 2014). In this scenario, the evolution of lifespan would respond to intraspecific density-dependent influences, with intraspecific competition for resources selecting against long lifespan in dense populations (Longo et al. 2005, Bassar et al. 2013, Mitteldorf and Martins 2014). Our findings provide a mechanistic basis to test this new scenario, as fatty acid characteristics may be one of the links between extrinsic mortality or population density and lifespan. That is, fatty acid chain length and degree of saturation may determine fitness outcomes after the action of extrinsic mortality or population density, hence being the target elements in the evolution of lifespan. Future studies should explore these possibilities. On the other hand, our study was made with birds and now it will be necessary to investigate if our evolutionary findings can also be applied to mammal species. Although any potential parallelism with humans should not be made at this stage, the future challenge will be to determine if these findings in birds can be used to understand longevity variation in humans, which will represent a new avenue for studying the evolution of lifespan in which fatty acid chain length should play a relevant role.

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Table 1. Fatty acid composition variables predicting maximum lifespan in two partial least squares regression (PLSR) models. Predictor weights (i.e. the contribution of each predictor variable to the PLSR component) and percentage of variance in maximum lifespan explained by the PLSR models are shown. When regression coefficients are statistically significant ($P < 0.05$), predictor weights are marked in bold. Reduced models refer to those made excluding species with sample size of one individual. Phylogeny refers to a number of phylogenetic eigenvectors (73 in full models, 50 in reduced models) used as predictors to account for phylogenetic effects, but only the predictor weight for the first eigenvector is shown. See Materials and methods for a definition of abbreviations.

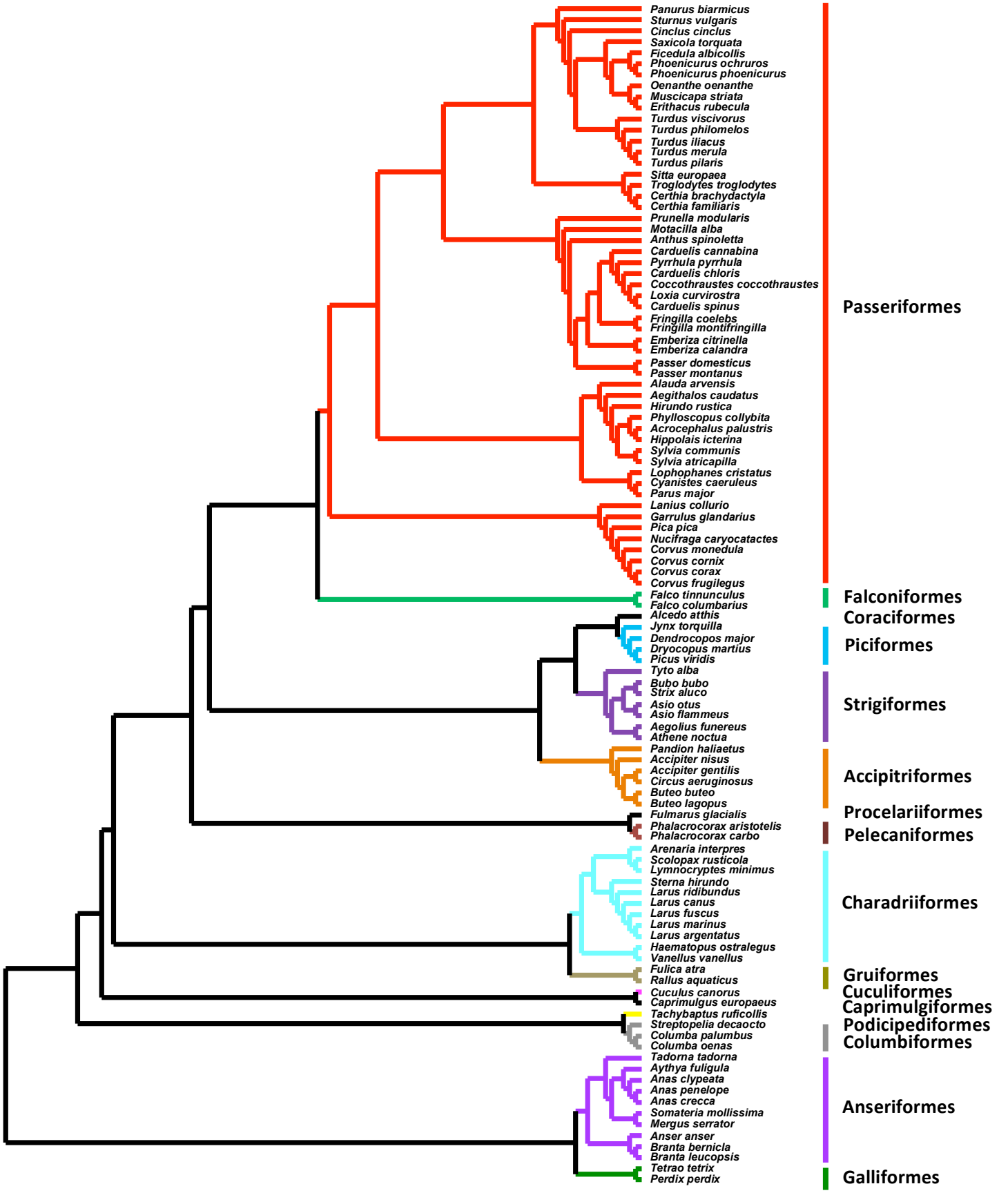
PLSR 1	Full model	Reduced model
Body mass	**0.51**	**0.53**
ACL	**0.28**	**0.25**
SFA	-0.16	-0.11
UFA	0.18	0.13
MUFA	**0.17**	**0.17**
PUFA	**-0.02**	**-0.06**
PUFAn-3	0.04	-0.00
PUFAn-6	**-0.04**	**-0.05**
DBI	**0.19**	**0.16**
PI	**0.18**	**0.16**
AI	**-0.22**	-0.27
Phylogeny	0.28	**0.42**
% variance explained	65.7	71.9

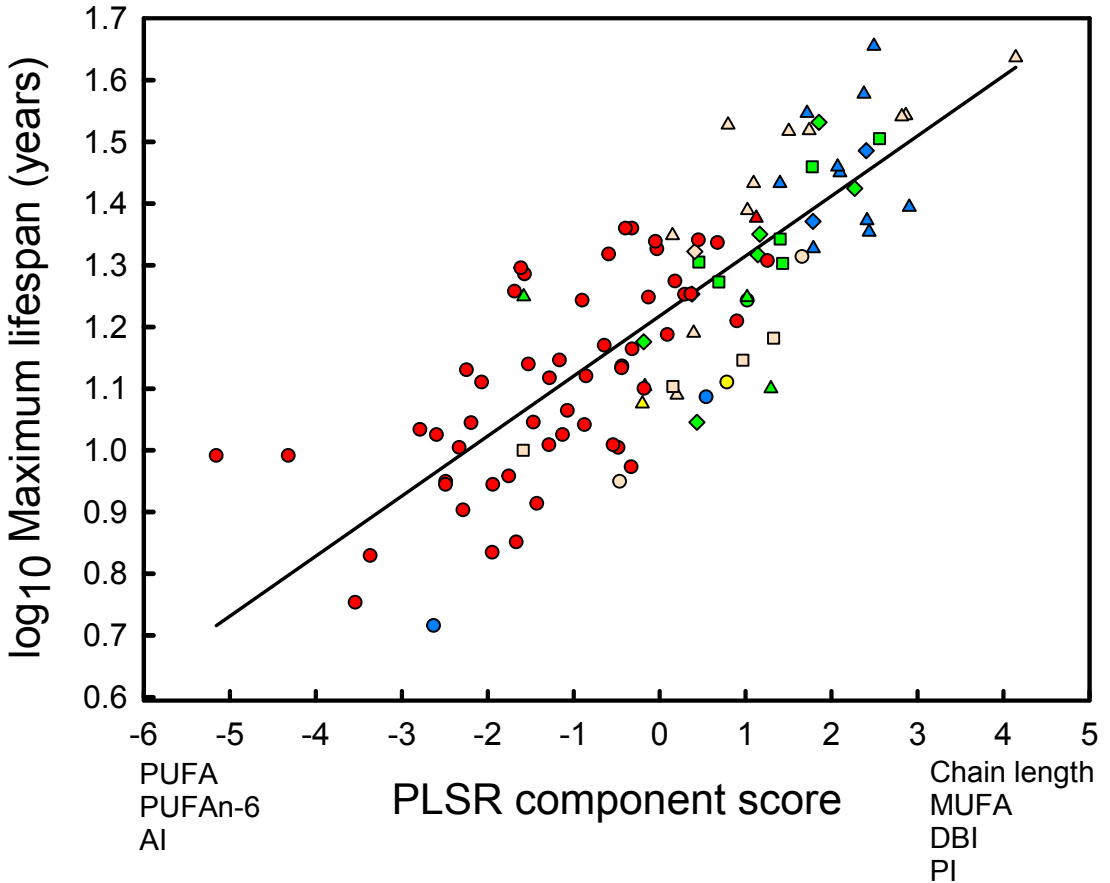
PLSR 2	Full model	Reduced model
Body mass	**0.42**	**0.44**
C14:0	-0.15	-0.11
C14:1	-0.04	0.01
C16:0	-0.16	-0.13
C16:1n-7	-0.01	0.03
C18:0	0.08	0.09
C18:1n-9	**0.10**	0.09
C18:2n-6	**-0.24**	**-0.22**
C18:3n-3	-0.13	-0.17
C18:4n-3	0.06	0.01
C20:0	0.16	0.15
C20:1n-9	**0.25**	**0.21**
C20:2n-6	0.12	0.08
C20:3n-6	**-0.05**	-0.09
C20:4n-6	0.25	0.30
C20:5n-3	0.04	0.02
C22:0	0.05	0.03
C22:1n-9	**0.14**	0.16
C22:4n-6	**0.33**	**0.31**
C22:5n-6	**0.14**	0.08
C22:5n-3	0.12	0.06
C22:6n-3	0.06	0.02
C24:0	0.01	0.05
C24:1n-9	0.12	0.15
C24:5n-3	0.00	-0.05
C24:6n-3	0.09	0.20
Phylogeny	**0.24**	**0.34**
% variance explained	64.6	67.2

Legends to figures:

Fig. 1: Phylogenetic hypothesis used in the study. Avian taxonomic orders are grouped by colours.

Fig. 2: Relationship between maximum lifespan and partial least squares regression (PLSR) component scores. Predictor names (excluding the number of recoveries, phylogenetic eigenvectors and body mass for the sake of simplicity) below the partial least squares regression component indicate which side of the axes increased with increasing values. The regression line is shown. Samples are grouped in taxonomic orders by colour codes: blue circles: Galliformes, blue triangles: Anseriformes, blue squares: Procelariiformes, blue diamonds: Pelecaniformes, green circles: Podicipediformes, green triangles: Columbiformes, green squares: Accipitriformes, green diamonds: Strigiformes, yellow circles: Cuculiformes, yellow triangles: Caprimulgiformes, pink circles: Gruiformes, pink triangles: Charadriiformes, pink squares: Piciformes, pink diamonds: Coraciformes, red triangles: Falconiformes, red circles: Passeriformes.


Panurus biarmicus
Sturnus vulgaris
Cinclus cinclus
Saxicola torquata
Ficedula albicollis
Phoenicurus ochruros
Phoenicurus phoenicurus
Oenanthe oenanthe
Muscicapa striata
Erithacus rubecula
Turdus viscivorus
Turdus philomelos
Turdus iliacus
Turdus merula
Turdus pilaris
Sitta europaea
Troglodytes troglodytes
Certhia brachydactyla
Certhia familiaris
Prunella modularis
Motacilla alba
Anthus spinoletta
Carduelis cannabina
Pyrrhula pyrrhula
Carduelis chloris
Coccothraustes coccothraustes
Loxia curvirostra
Carduelis spinus
Fringilla coelebs
Fringilla montifringilla
Emberiza citrinella
Emberiza calandra
Passer domesticus
Passer montanus
Alauda arvensis
Aegithalos caudatus
Hirundo rustica
Phylloscopus collybita
Acrocephalus palustris
Hippolais icterina
Sylvia communis
Sylvia atricapilla
Lophophanes cristatus
Cyanistes caeruleus
Parus major
Lanius collurio
Garrulus glandarius
Pica pica
Nucifraga caryocatactes
Corvus monedula
Corvus cornix
Corvus corax
Corvus frugilegus
Falco tinnunculus
Falco columbarius
Alcedo atthis
Jynx torquilla
Dendrocopos major
Dryocopus martius
Picus viridis
Tyto alba
Bubo bubo
Strix aluco
Asio otus
Asio flammeus
Aegolius funereus
Athene noctua
Pandion haliaetus
Accipiter nisus
Accipiter gentilis
Circus aeruginosus
Buteo buteo
Buteo lagopus
Fulmarus glacialis
Phalacrocorax aristotelis
Phalacrocorax carbo
Arenaria interpres
Scolopax rusticola
Lymnocryptes minimus
Sterna hirundo
Larus ridibundus
Larus canus
Larus fuscus
Larus marinus
Larus argentatus
Haematopus ostralegus
Vanellus vanellus
Fulica atra
Rallus aquaticus
Cuculus canorus
Caprimulgus europaeus
Tachybaptus ruficollis
Streptopelia decaocto
Columba palumbus
Columba oenas
Tadorna tadorna
Aythya fuligula
Anas clypeata
Anas penelope
Anas crecca
Somateria mollissima
Mergus serrator
Anser anser
Branta bernicla
Branta leucopsis
Tetrao tetrix
Perdix perdix
Passeriformes
Falconiformes
Coraciformes
Piciformes
Strigiformes
Accipitriformes
Procelariiformes
Pelecaniformes
Charadriiformes
Gruiformes
Cuculiformes
Caprimulgiformes
Podicipediformes
Columbiformes
Anseriformes
Galliformes


log10 Maximum lifespan (years)
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
-6
-5
-4
-3
-2
-1
0
1
2
3
4
5
PUFA
PUFAn-6
AI
PLSR component score
Chain length
MUFA
DBI
PI